Kentucky

Agricultural Experiment Station

University of Kentucky

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1. Burning and Non-burning Strains of Tobacco Mosaic.
2. The Ring Symptom of Virus Diseases of Plants.
3. Mosaic from Tobacco One to Fifty-two Years Old.

BULLETIN NO. 361

(RESEARCH BULLETIN)

Lexington, Ky.

December, 1935

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BULLETIN NO. 361

(RESEARCH BULLETIN)

1. Burning and Non-Burning Strains of Tobacco Mosaic.

W. D. VALLEAU and E. M. JOHNSON

Mosaic burn is a disease of tobacco which affects some of the leaves of mosaic plants which are without mosaic patterns. For this reason infections at topping time may cause extensive necrosis of the upper leaves which the grower is unable to associate with the mosaic disease. Many plants which have mosaic develop no burn, which tends to add to his confusion as to the cause of burn.

Attemps to produce mosaic-burn in the field during the summer of 1933, by the use of known strains of the tobacco mosaic virus, failed completely, altho burning occurred in naturally affected plants in near-by rows. With the thought that only certain strains of the mosaic virus cause burning, samples of mosaic-burned plants were collected from the field. Plants in the greenhouse were then inoculated during the fall, in comparison with plants inoculated with non-burning strains which had been tested in the field. The results were as expected; mosaic-burn appeared in the plants inoculated from burned plants, but not in those inoculated with the non-burning strains. In the summer of 1934, four rows of tobacco in the field were inoculated with two non-burning strains, severe mosaic and mild mosaic; and two burning strains, one collected several years ago from a field in which numerous plants were badly burned, and one collected in 1933 from a mosaic-burned plant. The plants were inoculated July 30, when about half grown, by rubbing the tip of one leaf of each plant between contaminated fingers. The

first difference observed was that the area rubbed with the mosaic-burning virus developed typical mosaic burning. The non-burning strain had caused no local symptoms many days after inoculation. Necrosis in the inoculated areas was not the same as the local necrotic lesions produced on *Nicotiana glutinosa* by any of the tobacco mosaic viruses and on some varieties of tobacco by certain viruses, as the variety used, Kentucky White Burley No. 5, is not one in which local lesions of this type develop.

Twenty-one days after inoculation observations were made on the inoculated plants. The results were as follows:

	Number of plants with		
	Severe burn	Slight burn	No burn
Row 1. Mosaic-burn virus	93	0	8
Row 2. Severe-mosaic virus	0	7	97
Row 3. Mosaic-burn virus	94	0	1
Row 4. Mild mosaic virus	2	0	106

The plants grew in a field where there had been some natural spread of mosaic from weed hosts, which may account for the discrepancies in the table. The plants in the mosaic-burn rows were so severely burned as to be worthless, whereas the only injury to the plants in the other two rows was distortion, stunt of growth, and the usual injury to quality caused by mosaic.

Extensive study has not been made of the strains of the mosaic virus which cause burn, but there is sufficient evidence to show that there are several burn strains which differ in symptoms. For example, in 1935, a row of White Burley tobacco was inoculated with ten strains of mosaic-burning virus from widely separated places. Five plants were inoculated with each strain, and 10 plants were inoculated with a non-burning strain from a field in which no necrosis whatever had developed in numerous infected plants. Mosaic burn appeared in nearly every plant inoculated with a burning strain, but there were distinct differences in the degree of burning produced by different strains.

The non-burning strain caused no necrosis. In 1935, studies on mosaic burn were continued, to determine the relative resistance of varieties of tobacco to mosaic burn and to determine which leaves of inoculated plants were subject to burn.

Figure 1. Mosaic burn on White Burley tobacco. The type of spotting on the leaf to the left is that commonly produced on the younger leaves, while on the right is the type which develops on the older leaves. This type is usually preceded by extensive chlorosis of the leaf, which has a tendency to follow the larger veins, as illustrated on the right half of this leaf. It is evident that the tobacco mosaic virus may, contrary to the commonly accepted view, cause symptoms in well-developed leaves.

SUSCEPTIBILITY AND RESISTANCE OF TOBACCO VARIETIES TO MOSAIC BURN

Five plants each of thirty-five White Burley varieties or hybrids, 22 varieties or hybrids of dark air-cured and dark fired tobacco, and 11 F_1 hybrids of Kentucky tobaccos with Ambalema, and plants of Ambalema, were inoculated with a burning strain of the mosaic virus. All these except Ambalema developed readily-observable mosaic burn. Five plants of each variety were inoculated with a similar severe mosaic of a non-burning strain, none of which developed burning. Two other mosaic strains,

white mosaic and an undescribed strain of ring mosaic, also were used but neither caused burning. There was some indication that certain varieties of White Burley and dark tobacco were somewhat more resistant to burning than others but the differences were slight and probably have no significance. The F_1 hybrids between White Burley or dark tobacco and the mosaic-resistant Ambalema, inoculated with the four mosaic strains which became systemically infected, developed mosaic typical of the strain used but of a milder, less distorting character. Mosaic burn developed to a slight degree in each plant inoculated with the burning strain but in no other plant. It may be concluded from these studies that, with the exception of Ambalema, there does not exist, in the varieties tested, a high enough degree of mosaic burn resistance to be of value in control of this phase of mosaic.

LEAVES AFFECTED BY MOSAIC BURN

Mosaic burn is evidently an invasive symptom (Figure 1) as it appears to be limited almost entirely to tissue young enough to be invaded rapidly by the virus, but does not occur on leaves which have completed growth at the time of inoculation or in leaves produced subsequently to the appearance of mosaic symptoms in the growing point. A record was made of the number of leaves on each of 109 plants of White Burley which developed mosaic burn in the field, following inoculation with the burning virus. The results were as follows:

Number of leaves showing mosaic burn 22 days after inoculation with a mosaic-burning virus.

No. of burned leaves per plant	No. of plants
0	5
1	1
2	13
3	23
4	33
5	25
6	8
7	1
Total	109

The leaves which burned were only partially developed at the time of inoculation but old enough so that mosaic patterns did not develop in them.

To determine to what extent burn occurred on leaves with mosaic patterns a record was made 22 days after inoculation of the number of plants bearing leaves with patterns and burn. The results were as follows:

No. of leaves with patterns and burn	No. plants
0	82
1	14
2	4
3	1
Total	101

Burn on leaves with mosaic patterns was usually confined to the older, patternless, distal portion of the leaf. Slightly younger leaves on which mosaic patterns extended to the tip never appeared to be typically burned altho much later necrotic spots occasionally developed.

THE EFFECT OF MOSAIC BURN ON QUALITY OF DARK TOBACCO AND OF WHITE BURLEY TOBACCO

Symptoms of mosaic burn range from a few nearly circular necrotic spots scattered over the surface of the leaf to numerous coalescing necrotic spots which finally kill the tissue of large areas of the leaf. In dark-fired tobacco a heavy leaf as free from blemishes as possible is desired. The top leaves of dark tobacco plants are the most valuable and are the ones most likely to burn if a burning strain of mosaic is spread during topping. Consequently, in fields of tobacco where a burning strain of the virus is present, extensive injury to the crop may follow topping.

White Burley tobacco is valued partly because of the good burning qualities of its chaffy lower leaves. These may be spotted with frogeye and other leaf spots, which cause a ragged appearance, and yet bring a good price on the market. The tip leaves are usually red and of an undesirable quality, and com-

mand only a low price. Anything which tends to produce a lighter colored, chaffy tip leaf adds to the value of the crop. At stripping, an improvement in quality has been noted in White Burley plants inoculated with a non-burning strain of tobacco mosaic at or just previous to topping. It was found, on stripping plants inoculated at topping time with a mosaic-burning strain, which caused extensive burning in the top leaves before cutting, that many of the burned leaves were lightened sufficiently in color and appeared so much like flyings (lower damaged leaves) because of burning that they could be graded as rather low-grade flyings, which command a decidedly higher price than red tips. Whether inoculation with a burning virus at topping time would be an advantageous practice is open to question; but the fact is worthy of record that infection with either a non-burning or a burning strain at topping time sometimes tends to improve rather than injure quality of White Burley tobacco. Hence it seems unnecessary to guard against mosaic spread in topping and suckering operations with this type of tobacco.

DISTRIBUTION OF BURNING STRAINS OF TOBACCO MOSAIC

Mosaic burn has been found widely distributed in Kentucky, altho no complete record of its distribution has been made. Probably it is as widespread as the mosaic disease. However, it does not occur in every field where mosaic is present. Some fields appear to be affected with only a single non-burning strain of mosaic; others with a single burning strain; and still other fields with mixed infections. On the Experiment Station farm at Lexington, tobacco grown near an old pasture always contains a mixture of many distinct strains of the virus, both burning and non-burning. As mosaic burning has been reported from other tobacco-growing regions of the United States, its distribution is probably coextensive with the tobacco industry.

2. The Ring Symptom of Virus Diseases of Plants.

E. M. JOHNSON and W. D. VALLEAU

In 1927, Fromme, Wingard and Priode (4) gave ample proof of the infectious nature of ringspot of tobacco. Wingard (30) transmitted the virus mechanically to 38 plants in 17 families and described symptoms on the various hosts. Henderson and Wingard (6) further established the identity of this disease by showing that the virus was inactivated by drying, that it passed a V filter, rarely survived a 1 to 10,000 dilution, and was inactivated by a temperature of 70°C for 3 minutes. Valleau (26) showed that this ringspot and a closely related one, yellow ringspot, were recognizable in plants which seemed to have recovered, by the abnormality and sterility of some of the pollen, and that both were seed borne. These and other studies have established tobacco ringspot as a definite and rather readily recognizable infectious disease of tobacco and other plants.

That the appearance of rings in plants is a good indication of the presence of a virus disease, not necessarily tobacco ringspot, is becoming increasingly evident. In 1931, Quanjer (16) considered the development of single or concentric circles alternating with green tissue and suggestive of the Liesegang phenomenon as a symptom of plant viruses and gave as examples a virosis of pea, of peony, tobacco ringspot, and a mosaic disease of the potato varieties, Arran Victory and Maaike. The writers have observed that ring and line patterns may occur as symptoms of various virus diseases of tobacco and other plants. During the last 10 years observations and records have been made of tobacco diseases caused by viruses with distinctly different properties but capable of producing rings, either as local symptoms on rubbed leaves or as transitory or permanent symptoms on uninoculated, invaded tissue. Some of these observations are reported in this paper.

LITERATURE REVIEW

Inoculation of tobacco plants with an extract from apparently healthy potato plants or those that showed mosaic symptoms, usually produced small chlorotic and necrotic rings (Figure 9 C) as has been shown by J. Johnson (9), Valleau (23), Valleau and E. M. Johnson (24), K. M. Smith (19), Burnett and Jones (2) and J. Henderson Smith (18). The latter also found that extract from potato affected with a mild mosaic (pre-

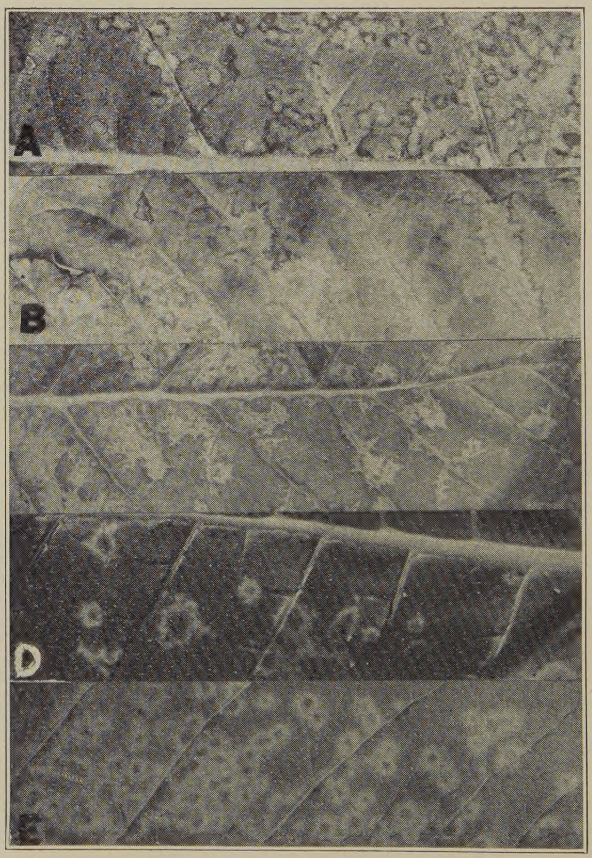

Figure 2. Leaves of White Burley tobacco affected with different viruses, showing ring symptoms. A. Ring mosaic. B. White tobacco mosaic. C. Yellow tobacco mosaic. D and E. Unidentified virus diseases, not true tobacco mosaics. A, B, and C from plants mechanically inoculated in the field. D and E from plants naturally infected.

sumably containing the virus present in most apparently healthy American potato varieties) when transferred to tomato, produced rings, in addition to necrotic spots and mottling, and showed a tendency to produce rings in *Datura stramonium, Nicotiana affinis, N. tabacum, N. sanderae, Solanum nigrum, S. dulcamara, S. villosum, S. nodiflorum, Hyocyamus niger, Nicandra physallodes, Petunia violacea, Capsicum annuum* and *Salpingoglossis sinuata.* In addition to the ringspot of tobacco described by Fromme and others, and J. Johnson's (9) ringspot, or in its milder form, mottle, from apparently healthy potatoes (Smith's X, Burnett and Jones' latent), E. M. Johnson (10) reported ring mosaic, yellow and white tobacco mosaics (Figure 2 A, B, C) the etch viruses (Figure 7 C), coarse etch, and cucumber mosaics (Figures 4, 5) as capable of producing ring patterns in tobacco. He also found that the cucumber mosaics produced small chlorotic and necrotic rings on inoculated leaves of bell pepper (*Capsicum annuum*) and chlorotic rings on rubbed leaves of beet (*Beta vulgaris*), and healthy-potato virus produced small chlorotic rings or necrotic circles on the leaves of beet, pepper, and pokeweed.

Samuel, Bald, and Pittman (17) stated that the bronzing of tomato foliage with spotted wilt sometimes occurred as zonate rings and rings were common and conspicuous on the fruit. Later Bald and Samuel (1) showed that this disease was transmissible to plants in 15 genera of 4 families, and mentioned the presence of concentric rings or zonate spots in tobacco, *Solanum sodomaeum, Physalis peruviana* and *Datura stramonium;* and faint yellowish rings in species of zinnia, aster and chrysanthemum. Smith (20) described a ringspot-like, naturally occurring disease of *Solanum capsicastrum* which, at the time, he thought was the ringspot of tobacco but which later he (21) identified as spotted wilt of tomato. He found that the spotted wilt virus produced rings in potato, tomato, tobacco, *Solanum capsicastrum, S. melongena, S. nodiflorum, Nicotiana glutinosa, Datura stramonium, Hyocyamus niger, Atropa belladonna, Petunia sp., Plantago major,* Dahlia and lupins. Altho the ring symptoms of spotted wilt on some of these hosts were similar

to those caused by tobacco ringspot it was shown not to be ringspot by being unable to live *in vitro* over 4 hours whereas ringspot may live 22 hours; ringspot is normally systematic in Petunia and spotted wilt is not; and ringspot is not mechanically transmissible to tomato as is spotted wilt.

Hamilton (5) noted that a naturally occurring virus disease, Hy. IV, of *Hyoscyamus niger*, after passage thru tomato,

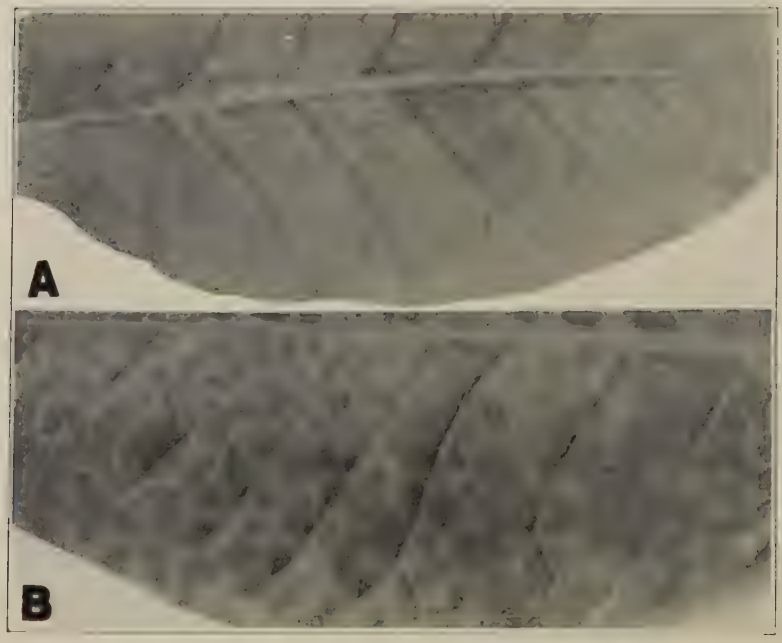

Figure 3. Rings and patterns in tobacco leaves affected with tobacco mosaics. A. A yellow ring tobacco mosaic in Ambalema tobacco. B. The same yellow ring mosaic in White Burley tobacco.

produced rings on tobacco not very different from those caused by Smith's X virus. It differed from the latter in not being mechanically transmissible to the potato varieties, Arran Victory, Arran Chief and Epicure.

Valleau (26) described a virus disease of Delphinium of which the conspicuous symptoms were yellow and chlorotic ring and line patterns in the leaves (Figure 11 C). The virus was

mechanically transmissible to tobacco where chlorotic and necrotic patterns accompanied a faint mottling. This virus did not cause pollen sterility; therefore was not tobacco ringspot. It was transmissible to cucumber where mosaic symptoms occurred, so it is possibly a strain of cucumber mosaic. Burnett (3) described a virus disease of Delphinium that may be the same as the one described by Valleau. Burnett's virus caused ring and line patterns in tobacco similar to those caused by the Kentucky strain (Figure 9 B).

Infectious chloroses of the rose were described by White (29) and Nelson (13) one of whom (Nelson) mentioned the occurrence of chlorotic patterns. A virus disease of rose, described by Valleau (26) (Figure 12 G), in which the leaves had faint chlorotic ring-like patterns, may be the same as the infectious chlorosis described by White and Nelson. Valleau (26) also described a virus disease of plum, transmissible to peach by budding (Figure 12 C) and a virus disease of apple (Figure 12 F). Chlorotic ring-like patterns were present in the foliage of the 3 species.

Miss Hogan (8) showed that a mosaic disease of sugar beet was readily transmitted to spinach by the aphid *Myzus persicae,* where rings, not unlike those produced by healthy potato on this host, appeared. The virus could not be mechanically transmitted to tobacco; therefore it was not the healthy-potato virus. Tobacco ringspot was mechanically transmitted to sugar beet where early symptoms were chlorotic and necrotic rings. Later, ringspot became systemic in sugar beet but the disease was unlike the mosaic of natural occurrence in this host.

A ringspot-like virus disease of red clover (probably yellow ringspot) was described by E. M. Johnson (11) (Figure 12 B) but he was unable to transfer the disease mechanically to red clover, tobacco, or garden beans. The latter two species are hosts of tobacco ringspot. Sweet clover was reported as a natural host of tobacco ringspot [Wingard (30)] but rings were not recorded as a symptom. Henderson (7) studied a naturally occurring virus disease of sweet clover in which rings were prominent. It was transmitted mechanically to tobacco where the

resulting symptoms were similar to those of the tobacco ring-spot earlier studied in Virginia (4). It differed from the latter

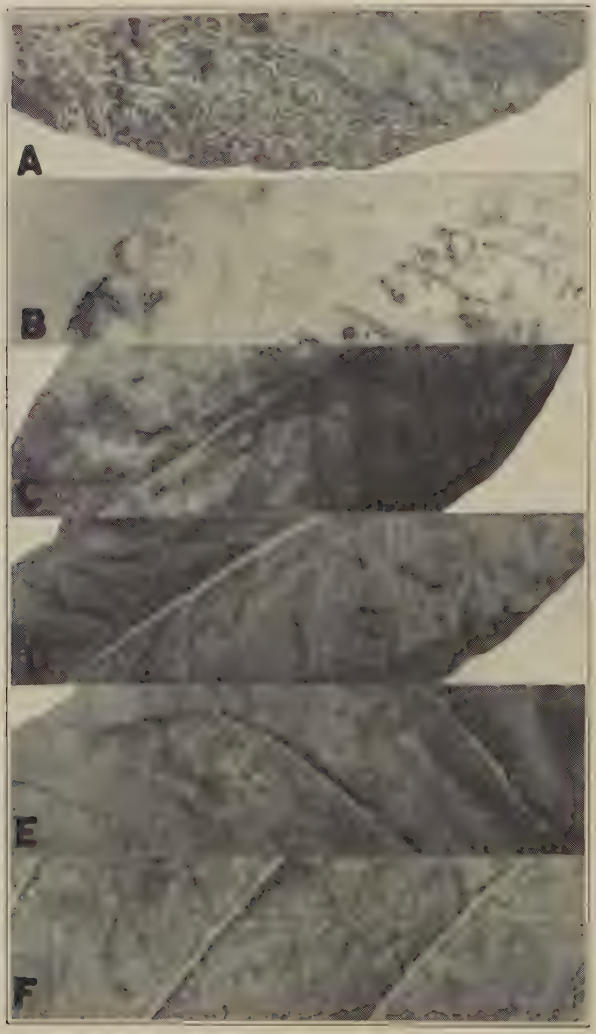

Figure 4. Leaves of White Burley tobacco from the field, showing ring symptoms caused by different viruses. A and B. White cucumber mosaic in leaves from the same plant. C. Delphinium virus in tobacco grown on the Experiment Station farm, St. Paul, Minn. D, E, and F. Chlorotic and necrotic zig-zag patterns and rings in leaves from tobacco plants suspected of having cucumber mosaic.

in that in tobacco there was no masking of symptoms; no development of immunity in the sense thought of by Price (14); and systemic infection without local spotting occurred in Petunia. Henderson believed this to be a strain of tobacco ringspot, but it may not be.

Wellman (27) found that celery virus 1, the cause of Southern celery blight, caused chlorotic and necrotic ring-like patterns in tobacco. In comparative inoculation and property studies with a cucumber mosaic, latent potato, tobacco ringspot and tobacco mosaic, he decided that the celery virus was different from any of these and was probably a new virus, yet it closely resembles the cucumber mosaic group of viruses.

OBSERVATIONS ON VIRUS DISEASES OF PLANTS WITH REFERENCE TO RING PRODUCTION

In the following pages the symptoms of some virus diseases of plants are described with especial attention to the occurrence of rings.

Tobacco mosaics. Three true tobacco mosaics, ring, yellow, and white (11), regularly produce rings in White Burley tobacco in the field and sometimes in Turkish tobacco in the greenhouse. In field plants ring mosaic produces definite rings composed of alternate bands of pale green to yellow tissue. Necrosis starts at the outer border of the ring and may involve the entire spot (Figure 2 A). In the greenhouse the rings are not often necrotic. This virus, which survives in dried plant material, causes streak of tomato and results in small rings in the leaves and fruit (25). White and yellow tobacco mosaics cause Liesegang-like rings of alternate bands of pale green and lemon yellow (yellow mosaic) to almost white (white mosaic) tissue on rubbed and unrubbed leaves. (Figures 2 B and C). McKinney (12) described rings on leaves of Connecticut Havana tobacco inoculated with a yellow mosaic from *Nicotiana glauca,* from the Canary Islands. White Burley tobacco plants affected with white tobacco mosaic also have almost white rings on the stems and capsules. Another ring mosaic, found in a White Burley plant, in 1934, produces yellow rings of the Liesegang

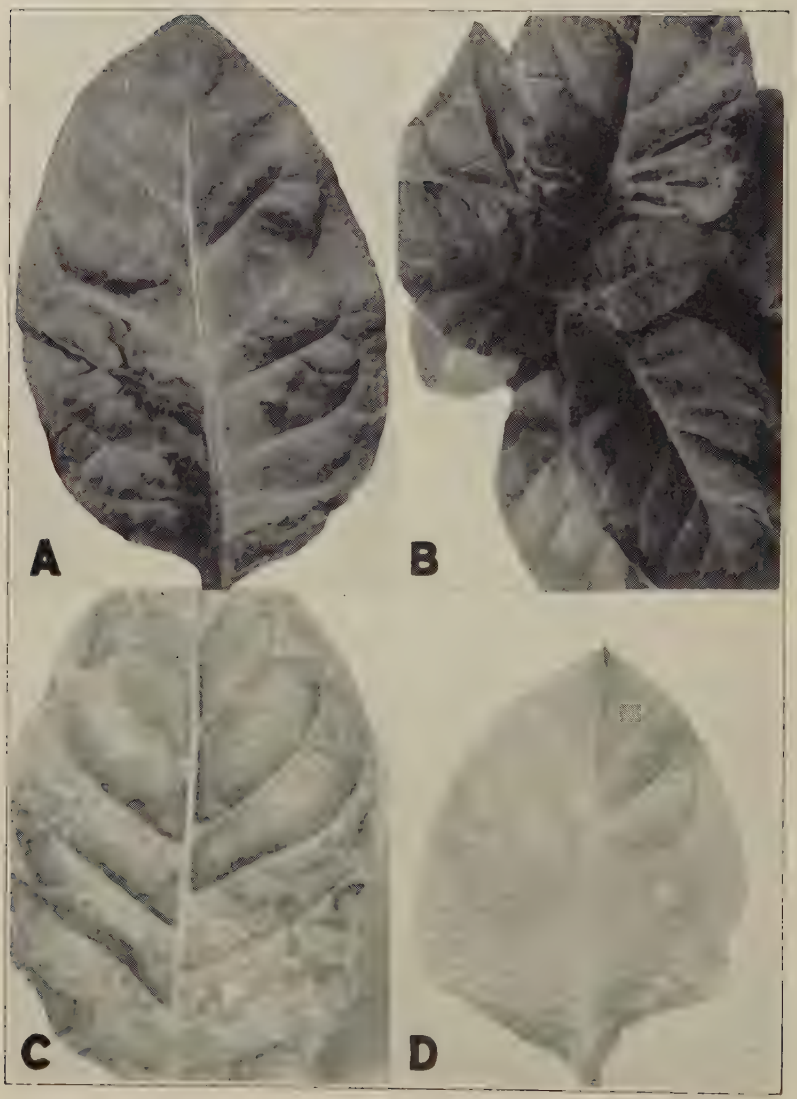

Figure 5. Ring patterns in leaves of Turkish tobacco grown in the greenhouse. A and B. Green cucumber mosaics. C. Mosaic from *Plantago major*. Necrotic rings usually develop in leaves of Turkish tobacco inoculated with this virus. D. Yellow cucumber mosaic. Yellowish rings with borders of green tissue characterize this virus disease.

type on White Burley, Turkish, and Ambalema varieties of tobacco (Figure 3 A and B). It differs from the ring mosaic described earlier, by the production of greater numbers of rings and the absence of distortion and mosaic mottling in the new leaves, which always accompanied the latter. In some field and greenhouse tests of tobacco mosaic strains certain ones produced necrosis (mosaic burning, Figure 1) in White Burley tobacco while other strains rarely resulted in necrosis. The necrotic strains usually produced yellowish rink-like patterns between and along the veins preceding necrosis (Figure 1). In a study of the viruses present in solanaceous weeds on the Experiment Station farm several strains of tobacco mosaic were found which caused no distortion but extremely faint chlorotic ring patterns on Turkish tobacco.

In 1935, following a period of cloudy weather from mid-February to mid-March, when the greenhouse temperatures got as low as 10°C several nights, and as high as 43°C during the day, 31 Turkish tobacco plants affected with various strains of green and yellow tobacco mosaics, developed necrotic rings on both rubbed and unrubbed leaves. The rings were of two types; necrotic circles 3 to 5 millimeters in diameter, and oak-leaf-like patterns generally associated with the midvein and primary veins (Figure 6). Necrotic circles, which appeared 18 days after inoculation were the first signs of infection on two plants, but in the others rings did not develop until 6 to 18 days after the appearance of mottling in the leaves of the growing points. Nine of the plants were inoculated with dried tobacco 52 years old and two each with dried tobacco 27 and 26 years old. All the other plants were inoculated with fresh extract from mosaic tobacco plants. Necrotic rings have been seen before on an occasional mosaic tobacco plant in the greenhouse but during the period noted nearly all mosaic tobacco plants in the greenhouse developt necrotic rings.

Cucumber mosaics. Nearly all the cucumber mosaics* studied are capable of producing chlorotic and necrotic ring and

* The term "cucumber mosaic" is used to include those viruses which produce a certain set of symptoms on tobacco, have a wide host range, and have either caused mosaic on cucumber or resemble strains that cause it.

line patterns on White Burley and Turkish tobaccos. White cucumber mosaic regularly produces concentric, mostly whitish, rings of various shapes and sizes in inoculated and uninoculated leaves of White Burley and Turkish tobaccos (Figure 4 A and B). The ring patterns often become necrotic, especially on lower leaves of tobacco plants in the field (Figure 4 F). Early symptoms of white cucumber mosaic are difficult to distinguish from those of white tobacco mosaic which usually causes greater injury and may result in death of the entire plant, if it is small when inoculated, while white cucumber mosaic usually results in dwarfing, the loss of some lower leaves, and the production of numerous white rings which may give a white cast to many leaves on a plant (Figure 4 B). The production of rings by cucumber mosaic was mentioned by Price (15) who found that his strain 1 produced yellow zonate rings on Turkish tobacco, *Nicotiana glutinosa,* and *N. langsdorfii* and strain 5 produced necrotic zonate rings in these hosts and in tomato.

Dr. J. G. Leach and Mr. J. M. Walter, Jr., grew White Burley tobacco plants at University Farm, St. Paul, in cooperation with this Station and sent the writers leaves of virus-affected plants for identification of the virus. Many of the leaves had chlorotic and necrotic ring-like patterns of various kinds. Inoculations to Turkish tobacco resulted in veinbanding, several distinct cucumber mosaic strains, and a virus identical with the one from Delphinium (Figure 4 C).** The delphinium virus is quite common in Delphinium in the vicinity of Minneapolis. Ring and line necrotic patterns developed in Turkish tobacco inoculated with cucumber mosaics from many of the Minnesota specimens.

White Burley tobacco plants, which have chlorotic and sometimes necrotic (Figure 4 D, E, F) ring and zig-zag line patterns are sometimes seen in the field. Such plants are usually suspected of having cucumber mosaic because of the distribution and kind of mottling in the newer leaves. Inoculations were made to Turkish tobacco plants in the greenhouse from the

** It is worthy of record that tobacco mosaic was not found in the entire planting. It suggests that tomato mosaic is commonly caused by other viruses in that region.

plants illustrated in Figure 4 D, E, and F, but no infection resulted. These may have been cucumber mosaics, because the latter are sometimes less readily transmitted to tobacco than

Figure 6. Leaves from a Turkish tobacco plant (12008) infected with a mild green tobacco mosaic. From left to right, top row, 1st, 3rd, 4th; bottom row, 5th, 6th, and 7th leaves from bottom of plant up. Leaves in the top row only were inoculated. The plant was exposed to unusually low greenhouse temperature during the invasion period. When the virus invaded the growing-point leaves, the plant "recovered" from "ringspot" and developed typical mild tobacco mosaic symptoms. Leaf 7 has symptoms typical of etch. The virus used was from dried tobacco passed thru 2 tobacco plants, then to *N. glutinosa* where it caused necrotic spots and stem streak. A bit of streak tissue was used to inoculate this plant (12008). The virus from this plant caused necrotic spots in another *N. glutinosa* plant.

some of the other viruses, or they may have been virus diseases that are not mechanically transmissible.

Other cucumber mosaics, a usual symptom of which is ring production, are illustrated in Figure 5 A, B and D. Ring-like

patterns with dark green centers always occur in plants affected with the cucumber mosaics illustrated in Figure 5 A and B. The leaf represented in Figure 5 D is a yellow cucumber mosaic which resembles white cucumber mosaic except that the ring patterns are some shade of yellow rather than white.

In Figure 7 B is shown a growing-point leaf of White Burley tobacco affected with what appears to be a cucumber mosaic. The chlorotic rings, especially in the tip third of the leaf, were the only symptoms observable in the naturally infected plant. When transferred to Turkish tobacco in the greenhouse small necrotic circles, broken ring-like necrotic line patterns, and circular, solidly necrotic spots appeared on both rubbed and unrubbed leaves. After a time pale green, almost yellow cucumber-mosaic-like mottling appeared in the new leaves. The virus was mechanically transmitted to Whippoorwill, New Era, and mixed cowpeas, where it caused systemic infection. The leaves of the New Era variety had a mosaic-like mottling while those of Whippoorwill and the mixed variety developed a yellowing spreading out from the veins or irregular yellow spots between the veins. The leaves of the last 2 varieties had a bronzed appearance. The disease was transferred from the New Era cowpea to Turkish tobacco where the symptoms were identical with those caused by inoculations from the naturally infected tobacco plant. Other strains of cucumber mosaic tested on the same varieties of cowpeas produced no visible symptoms. Price (15) found that several strains of cucumber mosaic caused necrotic spots on rubbed leaves of Black Eye and other varieties of cowpea as the only visible symptom. By single lesion transfers from cowpea to cowpea he obtained a yellow cucumber mosaic capable of causing systemic infection in cowpea. If the virus disease illustrated in Figure 7 B is a cucumber mosaic, and it appears to be because it produces circular chlorotic spots on jimson, it is probably the first recorded case of a cucumber mosaic of natural occurrence capable of causing systemic infection in cowpea.

The chlorotic and necrotic ring patterns in tobacco resulting from infection by cucumber mosaic and those from tobacco

mosaic are often not distinguishable. This is illustrated in Figure 8 by a comparison of A and C (cucumber mosaics) with B and D (tobacco mosaics). The similarity of the rings produced by white cucumber mosaic to those of white tobacco mosaic has been mentioned.

Figure 7. White Burley tobacco leaves from plants in the field naturally infected with virus diseases. A. An injurious virus disease of rare occurrence. Chlorotic and necrotic ring patterns are conspicuous on most leaves of affected plants. B. A virus disease like cucumber mosaic. This virus causes systemic infection in New Era cowpea. C. Chlorotic and necrotic rings in a leaf of tobacco affected with etch.

Etch Viruses. The viruses of the etch group produce small, necrotic circles, chlorotic spots, and rings with broken, necrotic borders, on White Burley (Figure 7 C), Turkish and dark tobacco varieties in the field. In the greenhouse, rings do not

often develop in dark tobacco. The etch viruses also produce the same types of rings in *Nicotiana rustica* plants.

Viruses from Mertensia virginica, and Plantago major. A virus from *Mertensia virginica,* when transferred to Turkish tobacco, caused chlorotic and necrotic rings on older leaves (Figure 9 D) and a cucumber-like mottling in the newer leaves. It is probably a strain of cucumber mosaic. A virus disease of *Plantago major* characterized by mosaic mottling in this host is readily transmissible to Turkish tobacco on which the first symptoms are small necrotic circles (Figure 5 C), not readily distinguished from the rings accompanying the cucumber mosaics, etch virus, healthy-potato virus, and others in tobacco. Chlorotic and necrotic rings, accompanied by mottling of the cucumber mosaic type, develop in unrubbed leaves. Usually the newer leaves show only mottling. This virus disease, which possibly belongs to the cucumber mosaic group, has been obtained from naturally infected White Burley plants in the field but it occurs rarely.

Unidentified tobacco viruses. Each year a few White Burley tobacco plants affected by a virus disease that causes severe injury are found on the Experiment Station farm. Usually fewer than 5 affected plants were seen in about 50,000 plants. Affected tobacco plants have a general chlorotic appearance, are dwarfed, and chlorotic and necrotic ring and zig-zag patterns are present on many leaves, especially the lower ones (Figure 7 A). Necrotic streaks develop along the midveins and laterals, usually starting on the lower leaves and progressing up the stem. Leaves with necrotic midveins sometimes have a fluted or tucked appearance. The necrosis gradually involves entire leaves and they fall, leaving at the top of the plant, only a few chlorotic leaves with necrotic ring patterns. Dead, brownish, sunken areas appear on the stem. Attempts to transmit this virus to tobacco mechanically were unsuccessful but it may be transmitted by grafting. A growing point of a naturally infected plant was cleft grafted on a White Burley tobacco plant in the greenhouse. The cion formed a union with the stock on one side but died in about 2 weeks. After 29 days suckers on the

stock plant showed symptoms typical of the disease in the field. The identity of this virus is not known, but it has much in common with tomato spotted wilt.*

Figure 8. Leaves of Turkish tobacco from the greenhouse, showing similarity of rings caused by tobacco and cucumber mosaics. A and C. Tobacco mosaics. B and D. Cucumber mosaics.

What appeared to be a virus disease of tobacco was observed by the junior writer in Bradfordsville, Kentucky, on September 28, 1923. A very high percentage of the plants were

* What appears to be the same disease was recently described by J. J. Johnson, Phytopathology 26: 285, 1936.

affected. The growing points of the plants, many of which were affected late in the season, were stunted and yellowish and some of the leaves had circular chlorotic areas or numerous small necrotic spots. The suckers were composed of distorted

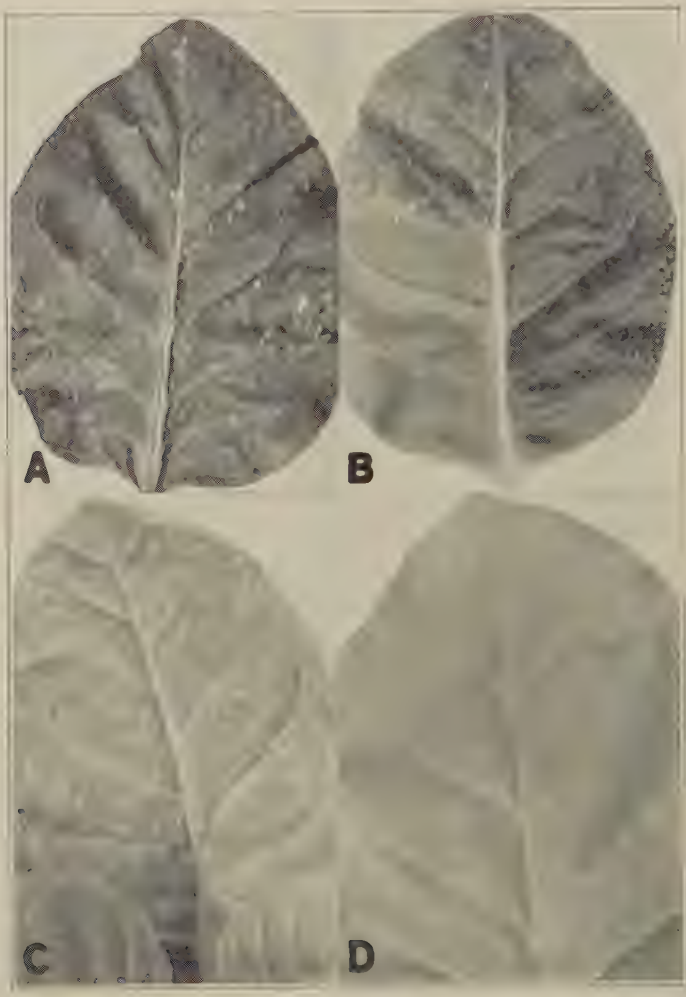

Figure 9. Leaves of Turkish tobacco from the greenhouse, showing [illegible] horsenettle (*Solanum carolinense L.*), Carmi, Illinois. B. Virus from Delphinium. C. Healthy potato virus (latent, X) from Irish Cobbler potato. [illegible]

leaves. Sunken necrotic areas were present on the stem and ring-like patterns were present in the pith (Figure 10 A, B). This disease has not been seen again and nothing is known of its transmissibility.

RINGS ON SPECIES OTHER THAN TOBACCO

Peony mosaic. The mosaic of peony, as described by Whetzel (28) often occurs in peony plantings in Kentucky but seems to cause little damage. The most striking symptom is the rings consisting of alternating bands of pale green to almost white tissue (Figure 11 A).

Lilac. In 1930, two lilac shrubs which had a general chlorotic appearance visible some distance away, were observed on the campus of the Agricultural College at University Farm, St. Paul, Minnesota. Lemon-yellow ring patterns, sometimes entirely covering a leaf, were conspicuous on some but not all shoots of the plant (Figure 11 B). Attemps to transmit the virus to tobacco were not successful. The shrubs were seen again in June, 1935. Again leaves of only certain branches bore patterns.

Pepper. Bell pepper plants inoculated with cucumber mosaics develop small, concentric, pale-green to almost yellow rings on rubbed and unrubbed leaves. Zonate patterns, sometimes of the oak-leaf type, may develop along the veins of the lower leaves. Pepper plants inoculated with the healthy-potato virus develop chlorotic rings about $\frac{1}{8}$ inch in diameter on rubbed leaves but these plants also develop a mottling not observable with the cucumber mosaics. Certain of the tobacco mosaics produce rings in foliage and fruit.

A virus-like disease was observed in bell pepper plants on the Station farm. Pale yellow to almost white rings of various sizes, somewhat like the rings in mosaic peony plants, were present in the leaves (Figure 12 A) and fruit. The disease was not transmitted to tobacco by rubbing. A similar virus disease was observed in pepper at Ordway, Colorado.

Potato. A virus from *Plantago major* when transferred to potato seedlings caused zonate, chlorotic spots which gradually

Figure 10. An injurious disease of White Burley from Bradfordsville, Ky., suspected of being a virus disease. Left. Ring patterns in the pith and distorted suckers are shown. Right. Dryish, sunken areas on the stem and chlorotic, sometimes necrotic, ring-like areas in the leaves were common symptoms.

became necrotic. Single leaflets may be almost entirely covered with concentric, chlorotic and necrotic spots (Figure 12 D).

New England Aster. A plant of the New England aster (*Aster novae-angliae*) with ring-like chlorotic patterns associated with the veins (Figure 12 E), was found by the junior author at Elk Lick Falls near Lexington. This appears to be a virus disease, but it has not been studied.

Sweet Clover. Plants of yellow sweet clover, *Melilotus officinalis,* in this area, either in fields or waste places, are often affected with at least two and possibly more virus diseases. One of these is of the mosaic type, symptoms of which are dwarfing, mottling, chlorosis and the production of an abnormal number of leaves. A ringspot-like virus disease occurs more often than the former. Symptoms of this disease on the leaflets are small rings, either solidly chlorotic or chlorotic with a normal green center and large, irregular, zig-zag, chlorotic, sometimes necrotic, patterns associated with the veins. The entire plant has a general chlorotic appearance and may be dwarfed. When the disease appears in an area, most of the plants are affected. Aphids are always numerous on sweet clover and may transmit the disease. The disease was mechanically transmitted to healthy sweet clover seedlings but a few attempts to transmit it by this means to tobacco were not successful. This may be the same disease of sweet clover described by Henderson (7) but he had no difficulty in transmitting the disease to tobacco.

DISCUSSION

Ring patterns of various types occur so often in plants affected with virus diseases that their appearance in a plant is almost always indicative of the presence of a virus disease. Rings may occur as the only symptom or as one of several symptoms of virus diseases of many species of plants. It is also known that rings, accompanying unlike virus diseases of certain species, may be so similar as to be indistinguishable. Necrotic oak-leaf patterns, indistinguishable from those of ringspot, sometimes develop on mosaic tobacco plants in the greenhouse. Usually other symptoms of diagnostic value accompany the rings, but in some

cases property and host range studies may be necessary to place the virus in its proper group. For example, spotted wilt of tomato may be indistinguishable from tobacco ringspot on some hosts, yet the viruses have different properties and a host range

Figure 11. Chlorotic rings in leaves of plants affected with virus diseases. A. Peony. B. Lilac, University Farm, St. Paul, Minn. C. Delphinium.

that is similar but not identical. The tobacco mosaics and cucumber mosaics may produce rings that are not distinguishable, but on hosts susceptible to both, the mottling symptoms which follow tend to identify the virus. Even the mottling accompanying these two viruses may be so similar in dark varieties, and sometimes even in Burley, that property or host studies must be used to classify the virus. The ring symptom is almost as common as mottling, dwarfing, chlorosis, or distortion, any one of which alone is unreliable for diagnosis.

In another paper,* ring patterns are explained as being invasive symptoms of the ringspot virus. This explanation seems to be satisfactory for the numerous ringspot-like diseases discussed in the present paper. The difference between tobacco ringspot and other virus diseases of tobacco in which ring patterns occur is in the occupative symptoms. The ringspot virus appears eventually to occupy the growing point completely, and patterns do not develop. Tobacco mosaic, cucumber mosaic, and other viruses which commonly cause rings, appear to be in an almost constant state of invasion of developing leaves. Very early invasion produces ordinary mottling, while later invasion is more likely to cause ring and line patterns. In a paper on "localization",† an experiment is described which seems to illustrate the method of distribution of the tobacco mosaic virus in a tobacco plant. When a plant of the mosaic-resistant tobacco, Ambalema, was furnished, by grafting, with a medium in which white mosaic virus could multiply, the white mosaic virus was distributed rather evenly thru the developing leaves of Ambalema. This was evidenced by the development of small, necrotic rings and spots at the points where the virus particles became lodged. It seems obvious, in this case, that distribution of the virus was purely a mechanical process. If this is the usual method of distribution of viruses within the plant, then ring patterns scattered over developing leaves are easily understood. The points of lodgment of the virus particles are centers around which the virus multiplies. This causes development

* Kentucky Experiment Sta. Bul. 360, No. 1.
† Kentucky Experiment Sta. Bul. 360, pages 214-215.

of necrotic or chlorotic patterns in exactly the same way that they develop at the points of inoculation when the virus is mechanically transmitted. Leaves in which ring patterns develop are rarely distorted, suggesting late invasion.

It is difficult to evaluate the theories that have been offered for the Liesegang phenomenon resulting from the diffusion of

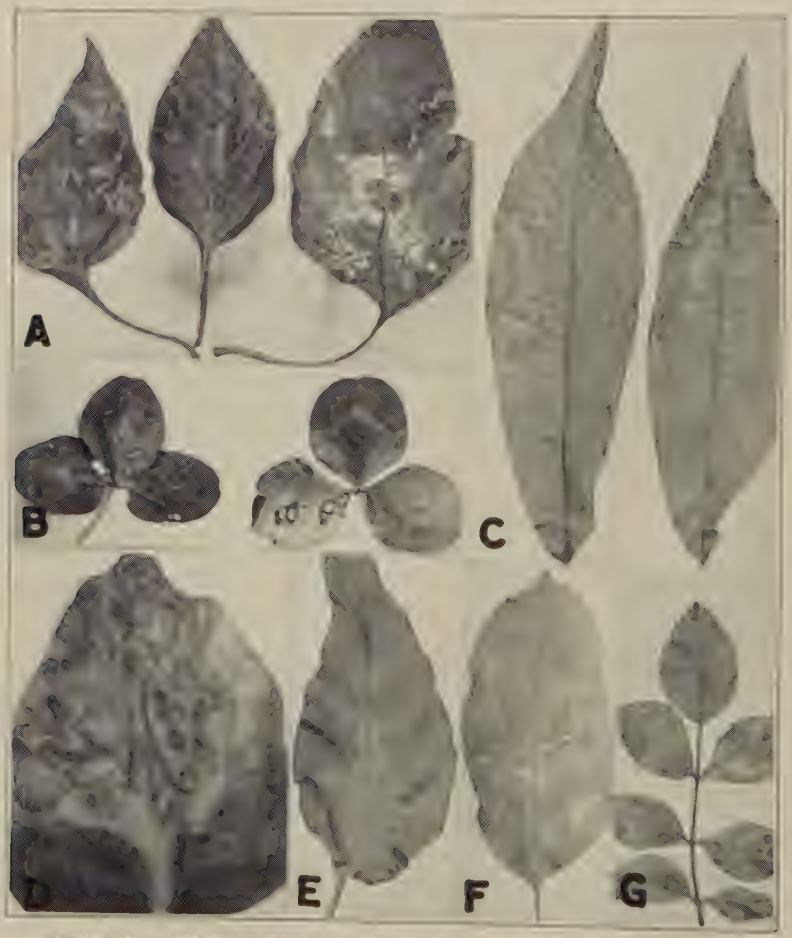

Figure 12. Chlorotic and necrotic rings in leaves of plants affected with virus diseases. A. Bell pepper. B. Red clover. C. Virus disease of peach transmitted from plum by budding. D. Potato leaflet affected with a virus from *Plantago major*. E. New England Aster (*Aster novae-angliae*). F. Apple. G. Rose.

solutions or sols in gels. The production of rings in plant tissues may be an analogous phenomenon, altho no biological application has been made of the Liesegang phenomenon. Virus particles follow the natural channels of solutions in the plant, in some cases, but they may also move by diffusion from cell to cell in addition to passing thru the plasmadesmids. Membranes thru which this diffusion takes place, if it occurs, are possibly colloidal gels, and there may be an interaction of chemical substances of virus and cell membranes analogous to the deposition of precipitates in gels. Alternating zones of necrotic and living tissue may be analogous to alternating colored rings and clear zones in gels.

SUMMARY

Chlorotic or necrotic rings may be present as the only definite symptom or as an accompanying symptom in plants affected with various virus diseases. Rings may be present as local symptoms on inoculated leaves or as transitory or permanent symptoms on uninoculated, completely or partially invaded tissue.

The following tobacco viruses capable of producing rings in tobacco are discussed: Ring tobacco mosaic (2 strains), yellow and white mosaic, cucumber mosaics, etch viruses, viruses from *Delphinium sp., Plantago major,* and *Mertensia virginica* (possibly cucumber mosaic), and two unidentified viruses.

Rings are also described as prominent symptoms in virus diseases of peony, lilac, delphinium, pepper, red clover, sweet clover, apple, plum, rose, and New England aster.

It is suggested that the ring symptom is not diagnostic for any specific virus disease because similar rings may occur in plants, especially tobacco, affected with such unlike viruses as tobacco ringspot, healthy potato, etch, tobacco mosaics, cucumber mosaics, and others. Usually other symptoms accompany the rings, which make classification possible, but sometimes property and host-range studies may be necessary for more exact classification.

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3. Mosaic from Tobacco One to Fifty-two Years Old.

E. M. JOHNSON and W. D. VALLEAU

In 1882, Mayer published the earliest known description of tobacco mosaic. The same year Mr. Orie LeBus, of Cynthiana, Kentucky, filled a packing case with buyers' samples of White Burley tobacco from the crop of that year. This case was not opened until the spring of 1935, when a liberal sample was obtained from which tobacco mosaic was induced in healthy plants without difficulty.

In 1927, Valleau and Johnson[1] showed that the virus in dry tobacco material 15 to 20 years old, was still infective. As so little is known of the exact nature of the virus, its ability to withstand very long periods of drying seems worthy of record. This paper presents the results of inoculation experiments with tobacco 1 to 52 years old.[2]

MATERIALS AND METHODS

The ground samples used in the earlier tests were retested in greater detail. They were furnished by the Chemistry Department of the Kentucky Agricultural Experiment Station. They had been stored in a basement room in screw-top glass jars which were labeled. The numbers and dates corresponded with those in the analysis books, so their ages may be considered authentic. The Chemistry Department samples and other samples known to contain mosaic, which were collected at various times from plants in the field and greenhouse, were kept in the laboratory in individual dated bags or envelopes. The sample from Mr. LeBus was protected from possible contamination with other tobacco.

[1] Valleau, W. D. and E. M. Johnson. Commercial tobacco and cured leaf as a source of mosaic disease in tobacco. Phytopath. 17 513-522. 1927.

[2] Several hands of dark tobacco 74 years old were obtained from Messrs. Jack and Joe Rogers, Versailles, Ky. The tobacco had a musty odor when received suggesting that it had been damp from time to time. Each hand was tested twice but mosaic failed to developed in a total of 64 inoculations.

Turkish tobacco plants,[3] for inoculation, were set with flamed forceps in steamed soil in steam-sterilized containers. The plants were grown 3 weeks or longer before inoculations were made and were kept in a vigorously-growing condition by additions of sodium nitrate solution. A small amount of dried tobacco material, not touched by the hands, was poured into a steamed aluminum dish or mortar containing 10 to 15 cc. of water. The material was ground with a pestle or a cloth swab on the end of a stick and allowed to soak for 15 to 60 minutes. This decoction was then rubbed on several leaves of Turkish tobacco plants with autoclaved cloth swabs, while the leaves were supported by clean pieces of cardboard held in the palm of the hand. The plants were never touched with the hands from the time of seeding until they were discarded. All the work of setting and caring for the plants and the inoculating was done by the writers.

TOBACCO MOSAIC FROM GROUND TOBACCO

In Table 1 are given the results of inoculations of Turkish tobacco with ground tobacco material, 24 to 39 years old. Two hundred and forty-one inoculations were made from 41 different samples of which 45 plants developed mosaic. Two of the samples were from entire plants, 2 from midveins, 28 from leaves, and 9 from stalks. Mosaic resulted from 1 sample from the entire plant, 1 from midveins, 9 from leaves, and 3 from stalks. At least 5 different mosaic strains, 3 green distorting, and 2 green non-distorting, resulted from these inoculations. Mottling was so mild in one of these that at times it was scarcely visible. Small, irregular, yellow blotches were present in many of the plants that developed mosaic.

TOBACCO MOSAIC FROM DRIED LEAVES OF TOBACCO PLANTS KNOWN TO CONTAIN MOSAIC

The results of the inoculations from known mosaic-diseased dried tobacco leaves, 1 to 15 years old, are given in Table 2.

[3] It was thought that *Nicotiana glutinosa*, because of its sensitivity to tobacco mosaic virus, would be an ideal test plant for small amounts of tobacco mosaic virus present in dried tobacco. The first 22 samples in Table 1 were tested on this species but only 3 samples, 3194, 17467, and 25617 resulted in 4 necrotic spots. When the same 22 samples were tested on Turkish tobacco, mosaic resulted from 13.

TABLE 1. TESTS FOR TOBACCO MOSAIC IN GROUND TOBACCO.

KAES Chemistry Dept. Analysis Number	Year of Collection	Plant Part	Age Years	Number Plants Inoculated	No. Plants Developing Mosaic	Days to First Symptoms	Mosaic Type*
3192	1895	Above ground parts	39	6	0	———	...
3194	1895	Above ground parts	39	6	4	8-11	GD
No number	1896	Leaf	38	6	6	11-13	GD
6141	1898	Midveins	36	6	0	———	...
11780	1902	Midveins	32	6	3	10	GD
17467	1906	Leaf	28	6	6	5-6	GD
17947	1907	Leaf	27	6	6	5	GD
17949	1907	Stalk	27	16	2	9-18	G
25613	1908	Leaf	26	6	0	———	...
25614	1908	Stalk	26	6	1	6	G
25615	1908	Leaf	26	6	2	5	G
25616	1908	Stalk	26	6	0	———	...
25617	1908	Leaf	26	6	4	7-13	G
25618	1908	Stalk	26	6	1	14	G
25620	1908	Stalk	26	6	0	———	...
25621	1908	Leaf	26	5	0	———	...
25622	1908	Stalk	26	5	0	———	...
25623	1908	Leaf	26	5	2	11-14	G
25624	1908	Stalk	26	5	0	———	...
25625	1908	Leaf	26	5	1	14	G
25627	1908	Leaf	26	5	1	11	G
25629	1908	Leaf	26	5	0	———	...
25631	1908	Leaf	26	5	2	11	G
25635	1908	Leaf	26	5	2	7-12	G
25636	1908	Stalk	26	5	0	———	...
25637	1908	Leaf	26	5	1	11	G
25638	1908	Stalk	26	5	0	———	...
Christian Co. 2	1909	Leaf	25	8	0	———	...
Christian Co. 4	1909	Leaf	25	8	0	———	...
Christian Co. 6	1909	Leaf	25	8	0	———	...
Christian Co. 8	1909	Leaf	25	8	0	———	...
Christian Co. 12	1909	Leaf	25	8	0	———	...
Christian Co. 14	1909	Leaf	25	8	0	———	...
Christian Co. 16	1909	Leaf	25	8	0	———	...
36636	1910	Leaf	24	3	0	———	...
36637	1910	Leaf	24	3	0	———	...
36638	1910	Leaf	24	3	0	———	...
36639	1910	Leaf	24	3	0	———	...
36641	1910	Leaf	24	3	1	14	G
36642	1910	Leaf	24	3	0	———	...
36644	1910	Leaf	24	3	0	———	...

* G = Green non-distorting. GD = Green distorting.

Eighty-four inoculations from 76 different specimens produced 79 cases of mosaic. All the tobacco mosaics, described in a previous publication,[4] 2 additional yellow mosaics, another white mosaic, and several undescribed green strains were represented in the samples tested. All strains, except ring mosaic, which produced no rings in these tests, survived unchanged in dried material for the periods indicated. With the method of inoculation used there was no evidence of loss of infectivity of the virus in dried samples as old as 8 years. There was a decrease in number of plants infected with mosaic from dried tobacco 10 to 15 years old but the percentage of infection was still high. The results indicate a gradual decrease in virus concentration with age, but show that drying is a safe, convenient, and economical method of preserving tobacco mosaic material for future studies.

TOBACCO MOSAIC FROM DRIED TOBACCO LEAVES 52 YEARS OLD

Thru the kindness of Mr. Orie LeBus, of Cynthiana, Kentucky, several pounds of White Burley tobacco, 52 years old, were obtained for tests. This tobacco consisted of buyers' samples from the 1882 crop bought by a tobacco firm of which Mr. LeBus was bookkeeper. This crop was grown the same year that Adolf Mayer[5] published the first description of tobacco mosaic. At the time of purchase, the hands of tobacco, with labels attached, were put into a box, stored in an outbuilding, and left undisturbed until 1935. The sample tested was obtained soon after the box was opened. There appears to be no question as to the age of the tobacco because Mr. LeBus has the original labels. Thirty tobacco plants, 10 at one time, and 20 three weeks later, were inoculated with decoctions made from a composite sample of inside leaves of several hands. In the first series of 10 inoculated plants, 6 developed mosaic in 8 to 12 days. Another developed in 18 days, a single yellow zonate spot on the margin of a rubbed leaf. A portion of this spot

[4] Johnson, E. M. Virus diseases of tobacco in Kentucky. Ky. Agr. Exp. Sta. Bul. 306. 1930.

[5] Mayer, Adolf. Over de mosaikziekte van de tabak. Woorloopige Meded. Landbouwk. Tijdschr. 359-364. 1882.

was removed with a cork borer and inoculations made to 3 Turkish tobacco plants all of which developed a yellow mosaic 6 days later (Figure 13 B). Yellow mosaic mottling, identical with that of these 3 plants, developed in the growing-point leaves of the plant with the zonate spot 6 days after the appearance of the spot. In the series of 20 inoculations, 11 plants de-

Figure 13. Strains of tobacco mosaic from dried tobacco. A. A mild green mosaic from No. 25623 from the 1908 crop. Mottling was almost invisible and there was no distortion. B and D. A yellow, and a green non-distorting mosaic from tobacco 52 years old. C. A green distorting mosaic from No. 3194 from the 1895 crop.

veloped mosaic in 6 to 10 days. All these were green, moderately distorted mosaics (Figure 13 D). One plant had numerous yellow spots on 2 leaves. Attempts to isolate a yellow mosaic by cork borer inoculations from single yellow spots directly to Turkish tobacco and thru *N. glutinosa* resulted in green mosaics accompanied by many yellow spots.

TABLE 2. LONGEVITY OF KNOWN TOBACCO MOSAIC VIRUSES IN DRIED LEAF TISSUE.

Age in Years	Number of Specimens	Number Plants Inoculated	Number Plants Developing Mosaic	Days to First Symptoms	Type of Mosaic*
1 to 2	6	6	6	4 to 7	GD1, GD2
2 to 4	32	32	32	4 to 9	G1, GD1, GD2, GD3, Y, W
6 to 8	25	25	25	4 to 14	G1, GD1, GD2, GD3, GD4, GD5
8 to 10	11	11	8	6 to 9	G2, GD2, GD3, GD4
10 to 15	2	10	8	6 to 18	G1, G2, GD2

* G1. Green mosaic, no distortion, no general chlorosis, mottling mild. G2. Green mosaic, no distortion, more chlorosis and mottling more distinct than in G1. GD1. Green distorting, distortion slight, no general chlorosis, mottling without blisters. GD2. Green distorting, distortion slightly more marked than in GD1, mottling as small, green blisters, leaf tips with a pinched appearance. GD3. Green distorting, more leaves distorted and more strikingly so than in GD2, many leaves much narrowed, green areas along veins blister like, no general chlorosis. GD4. Green distorting, lower leaves very narrow, sometimes strap-like and chlorotic, some leaves with irregular cut in margin, dark green areas blister-like, no general chlorosis. GD5. Green distorting, like GD4 as to distortion and blistering, but lower leaves have a speckled appearance due to even distribution of pale green tissue between the dark green areas. Y. Yellow tobacco mosaic. W. White tobacco mosaic.

During the latter part of February and early March there was a prolonged period of cloudy weather when the greenhouse temperatures fell as low as 10°C nights. Day temperatures were sometimes as high as 41°C. Necrotic oak-leaf rings and necrotic circles developed during this period on 13 of the plants that were affected with green mosaics. These plants were inoculated with 27 to 52-year-old dried tobacco (Figure 14).

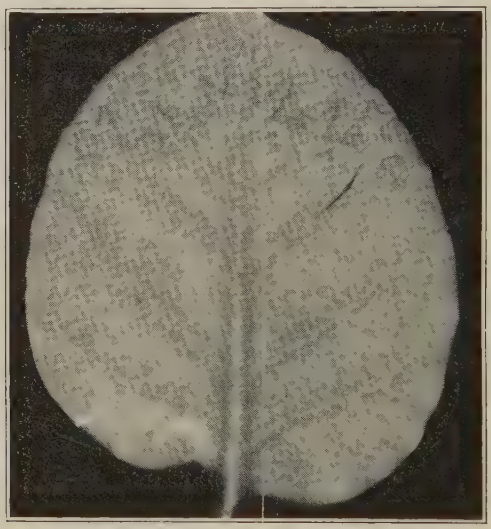

Figure 14. Direct print of leaf showing oak-leaf necrotic pattern. Leaf from a Turkish tobacco plant inoculated with extract from ground tobacco stalks, 26 years old (No. 25618). The growing-point leaves of this plant had a mild mosaic mottling. (Natural size.)

SUMMARY

Turkish tobacco plants never touched by hands were inoculated with decoctions made from dried tobacco material 1 to 52 years old. Forty-one ground samples 24 to 39 years old were used to inoculate 241 tobacco plants of which 45 plants, representing 17 samples, developed mosaic. At least 5 different strains of mosaic, recognizable by different degrees of severity of symptoms, were produced from the Chemistry Department samples.

Seventy-six samples, 1 to 15 years old, known to contain mosaic when collected, were used as inoculum for 84 tobacco plants of which 79 developed mosaic. There was no evident loss in infectivity of the mosaic virus in dried tobacco 8 years old. After 8 years there seemed to be a gradual decrease in the concentration of the virus. At least 12 strains of the mosaic virus were tested and all survived in dried tobacco.

Dried leaves from tobacco grown in 1882, the year Adolf Mayer published the first description of tobacco mosaic, were tested for tobacco mosaic virus. Of 30 plants inoculated with this material, 18 developed mosaic. Two strains, a yellow, and a green distorting type, appeared.

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